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Brief Communication

FREE RADICALS FROM PLASTIC SYRINGES

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Abstract—We have found that disposable sterile plastic syringes can leach free radicals into the fluids they hold. The free radical we observe appears to be a nitroxide (aminoxyl radical) because in aqueous solution it produces a 1:1:1 three line spectrum with a hyperfine splitting constant of 16.9 G.

Keywords—Free radicals, Syringes, ESR

INTRODUCTION

The role of free radical processes are now under investigation in many human health problems[1] and as these studies progress from biochemical to clinical the complexity of the studies and data interpretation increases. In addition, the equipment used to handle samples in a clinical setting is often different from that in a biochemical study. In this communication we report that sterile plastic syringes commonly employed in clinical settings can introduce free radicals into the samples.

MATERIALS AND METHODS

Syringes were sterile single-use plastic from Becton Dickinson and Co. (Rutherford, NJ). Over 20 syringes from this product line of varying sizes were examined, all yielding similar results. The 3-carboxy-proxyl and 4-amino-tempo were from Aldrich Chemical Co. (Milwaukee, WI). ESR spectra were collected with a Bruker ESP-300 electron spin resonance spectrometer using a TM_{110} cavity and a flat cell.

RESULTS AND DISCUSSION

Figure 1 (top) demonstrates the ESR spectrum of the contaminating free radical that arose when 2 mL of saline water was drawn into a new 10 ml sterile single-use syringe. To acquire the sample for the bottom spectrum of Fig. 1, the syringe was filled and emptied three times with saline before 2 mL of saline water was placed in the syringe and subsequently examined by ESR. This demonstrates that although the free radical species does partially wash out, it is stubborn and persists. Using 3-carboxy-proxyl as a standard, the concentration of the free radical observed in Fig. 1 (top) is ≈ 0.2 μM. This free radical was also observed in 5-mL and 30-mL syringes of this product line. The use of nonsterile syringes from this product line can also introduce this free radical, however, their concentration was much less (≈ 1/10 – 1/100 or less) than that seen in the sterilized syringes. Sterile syringes from different product lines, that

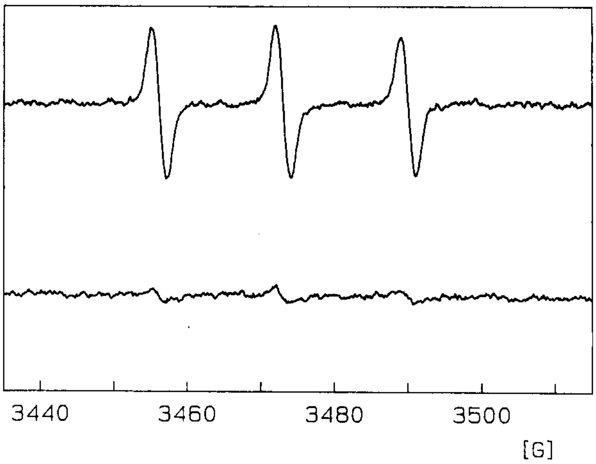

Fig. 1. *Top:* ESR spectrum of the "nitroxide" radical leached from a 10-mL sterilized single-use syringe; a^N = 16.9 G in saline. *Bottom:* Radical after flushing this syringe three times with 10 mL of saline. Instrument settings were: receiver gain, 5 × 10^5; mod. amp., 2 G; sweep rate, 80 G/168 s; time constant 0.66 s; power, 40 mW.

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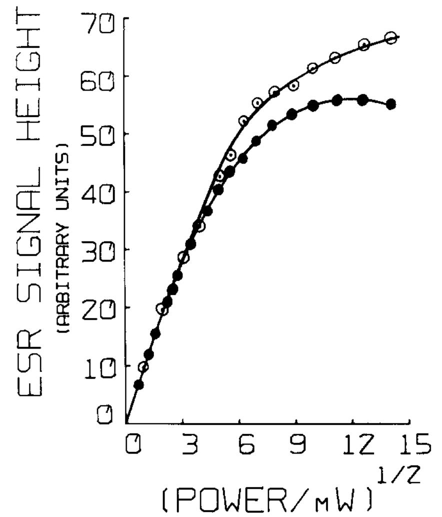

Fig. 2. Power saturation curve for the spectrum shown in Fig. 1 and for 3-carboxy-proxyl. ⊙ = syringe nitroxide, ● = 3-carboxy-proxyl (0.1 mM in 5 mM pH 7.4 phosphate buffer). Instrument settings were as in Fig. 1. The onset of saturation effects beginning at approximately 40 mW nominal power is typical of a freely tumbling nitroxide in aqueous solution. Also, the power saturation curve for 3-carboxy-proxyl, a five-membered ring nitroxide, was identical to that of 4-amino-TEMPO, a six-membered ring nitroxide, (0.1 mM in 5 mM pH 7.4 phosphate buffer), not shown.

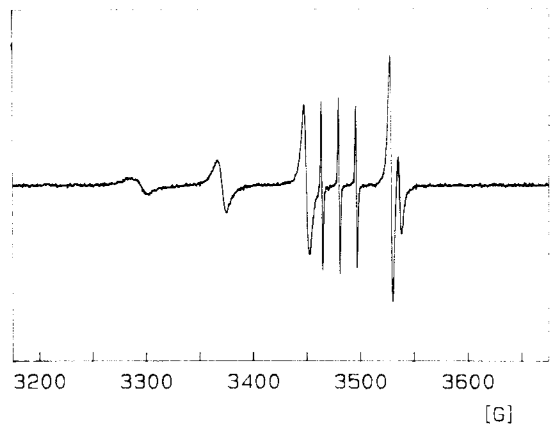

Fig. 3. ESR spectrum of the copper–(sulfur-compound) complex in room temperature chloroform solution. The sharp three-line spectrum centered at 3475 G is the "syringe nitroxide." Instrument settings were: receiver gain, 2.5 × 10^5; mod. amp., 1.0 G; sweep rate 500 G/671 s; time constant, 0.16 s; power 20 mW.

is, different composition, and glass syringes did not introduce this free radical.

This three line 1:1:1 signal is typical of a nitroxide free radical. If the radical is assumed to be a nitroxide, then $a^N = 16.9$ G in water: again, a parameter typical for a freely tumbling nitroxide radical. The radical can be extracted into organic solvents; in $CHCl_3$ $a^N = 13.9$ G. The power saturation curve, Fig. 2, is also typical of the saturation behavior of freely tumbling nitroxide free radicals.

Although the exact identity of this free radical has not been made, the evidence clearly points to a nitroxide radical. Nitroxide radicals are often synthesized by the oxidation of hindered amines. It may well be that radiation sterilization of the syringes brings about the oxidation of a hindered amine antioxidant, forming the observed radical.

In addition to the nitroxide radical described above, occasionally we have also observed an additional EPR active species in our Folch extracts of blood samples, Fig. 3. This species has previously been observed by Antholine et al.[2] and has been identified as a low molecular weight copper–(sulfur compound) complex similar to copper-dithiocarbamate, $g_{iso} = 2.044$ and $a_{iso} =$ 75 gauss. This species can arise when samples come in contact with such things as surgical gloves or rubber stoppers; the sample provides the copper, while the sulfur compound apparently leaches from the glove or stopper.[2] Beware!

Although these radicals can be avoided with appropriate choice of apparatus and care in sample handling, the possible infusion of the nitroxide radical into patients when using syringes in a clinical setting may be of concern. Additionally, hindered amines and nitroxides can serve as antioxidants, thus their presence, adventitiously, in biochemical and biological free radical studies could alter the course of the free radical processes being investigated. Therefore, we wish to caution researchers on the use of these materials in their free radical studies.

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